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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

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SEP 27 1994

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: **METHYL BROMIDE. ID #053201.** Evaluation of a Chronic Toxicity/Carcinogenicity Inhalation Toxicity Study in Mice (and Ancillary Short-Term Toxicity and Genotoxicity Studies) Conducted by the National Toxicology Program.

Tox. Chem. No.: 555
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CONCLUSIONS

TB-I has reviewed the National Toxicology Program (NTP) 2-year inhalation carcinogenicity study in mice (DER attached to this memorandum). Results are summarized below in the Executive Summary. In addition, this report contained short-term and target-organ toxicity studies in mice and rats (summarized in the Appendix to this memorandum) and genotoxicity tests (summarized in the attached DER) on methyl bromide.

EXECUTIVE SUMMARY: In a 2-year chronic toxicity/carcinogenicity study, 50 B6C3F$_1$ mice/sex/dose were exposed for 102 weeks to methyl bromide vapor at atmospheric levels of 0, 10, 33 or 100 ppm (0, 0.03876, 0.1279 or 0.3876 mg/l). In addition, 10 animals/sex/dose were included for interim sacrifice at 6 months and 10 at 15 months, and groups of 16 animals/sex/dose for neurobehavioral assessment at 3 month intervals. Exposures were conducted 5 days/week for 6 hrs/day. Exposure of all high dose animals was terminated after 20 weeks due to excessive mortality

in males, and males from the interim sacrifice and neurobehavioral groups were incorporated into the main group to ensure adequate survivors.

At 100 ppm, survival was markedly decreased in males (23% vs. 82%, controls, at termination). Clinical signs in males and females included abnormal posture, tremors, ataxia, limb paralysis and emaciation. Some neurobehavioral effects were also observed (including increased startle response, decreased general activity). Decreased mean body weight gain was also observed (-56% less than controls, males and -41% less than controls, females). Microscopic lesions included degeneration of the cerebellum (44%, males; 18%, females vs. 0% in controls), cerebral degeneration (16%, males, 3%, females vs. 0% in controls), chronic cardiomyopathy (54%, males, 58%, females vs. 8% and 2%, male and female controls), cardiac degeneration (45%, males and 12%, females vs. 2%, female controls), sternal dysplasia (15 - 20% vs. 0% in controls) and olfactory epithelium metaplasia or necrosis (2 - 9% vs. 0% in controls). **The LOEL for systemic toxicity in this study is 100 ppm (0.3876 mg/l), based on mortality (males), neurological signs, decreased body weight/weight gain and microscopic lesions in the brain, heart, sternum and olfactory epithelium. The NOEL for systemic toxicity is 33 ppm (0.1279 mg/l).**

There was no evidence of carcinogenic potential of methyl bromide. Although excessive toxicity occurred at 100 ppm, the dosing is considered adequate because (1) methyl bromide has a steep dose-time dependent mortality curve and (2) at 33 ppm, marginally lower mean body weight gain in females (-12%) and sporadic incidence of abnormal posture late in the study (1 male, 3 females) may represent a threshold NOEL.

This study is Core-minimum and satisfies the guideline requirement for a carcinogenicity study in mice (83-2b), despite some problems with the dose selection (see Discussion/Conclusions in DER). The study does not satisfy the guideline requirement for a chronic toxicity study (83-1a) in rodents due to study deficiencies including no clinical chemistry data, no urinalysis and no reporting of gross findings. However, although it was submitted for both 83-1a and 83-2b, a rodent chronic inhalation toxicity study is not required for methyl bromide.

Short-Term and Target Organ Toxicity Studies in Mice and Rats (additional information, not guideline studies): See attached Appendix for summary of results.

Genotoxicity Studies on Methyl Bromide (additional information, not guideline studies): Methyl bromide was mutagenic in Salmonella reverse gene mutation assays, mouse sister chromatid exchange assays (in vivo exposure) and mouse peripheral blood cell micronucleus assays (in vivo exposure).

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See attached DER for summary of results.

ACTION REQUESTED

On behalf of the Methyl Bromide Industry Panel, the Chemical Manufacturers' Association submitted for review a 2-year mouse inhalation chronic toxicity/carcinogenicity study (NTP report) on methyl bromide. This study was submitted to support reregistration of methyl bromide and fulfill Guideline 83-2b (inhalation carcinogenicity study in mice). The study report also contained 14-day and 13-week inhalation toxicity studies in mice and rats and target organ studies, which were summarized by TB-I to provide additional information on the toxicity of methyl bromide.

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APPENDIX: ADDITIONAL TOXICITY STUDIES CONDUCTED IN RODENTS IN THE NTP REPORT ON METHYL BROMIDE (NTP TR 385, MARCH, 1992)

[Information summarized - detailed review not conducted. Additional details on materials and methods of these studies is contained in the DER for the NTP mouse (inhalation) carcinogenicity study. The 14-day and 13-week mouse inhalation studies were conducted as dose range-finding studies for the 2-year study.]

14-Day Inhalation Study in Mice

Study Design: Ten B6C3F$_1$ mice/sex/dose were exposed to methyl bromide vapor by inhalation at 0, 12, 25, 50, 100 or 200 ppm (0, 0.0463, 0.09257, 0.1938, 0.3876 or 0.7751 mg/l) for 6 hrs/day, 5 days/week over 14 days. All animals were observed twice daily, weighed at 0, 5 and 114 days and necropsied. Clinical pathology was not performed and organ weights were not determined. Tissues from control animals and animals exposed to 100 and 200 ppm were examined microscopically.

Results: Mortality was observed at 200 ppm in males (9/10) and females (6/10) but not at 100 ppm or lower. Deaths occurred between Days 11 and 14. Clinical signs that appeared to be related to treatment were observed at 50 ppm and above and included paralysis, tremors and jumpiness (number of animals affected at each dose and severity of symptoms were not indicated). Blood was also observed in urine of mice in the 200 ppm dose group but incidence/frequency were not reported. No treatment-related decreases in body weight were observed; however, due to mortality, terminal body weight was not measured in 200 ppm animals. Except for minimal hyperemia of the lung, liver or urinary bladder in mice exposed to 200 ppm, microscopic lesions related to treatment were not observed. Individual animal data were not provided for this study.

13-WEEK INHALATION STUDIES

13-Week Inhalation Study in Mice

Study Design: Ten B6C3F$_1$ mice/sex/dose were exposed by inhalation to 0, 10, 20, 40, 80 or 120 ppm (0, 0.03876, 0.1550, 0.3100 or 0.4651 mg/l) methyl bromide vapor for 6 hrs/day, 5 days/week over 13 weeks. All animals were observed twice daily and body weights were measured weekly. Neurobehavioral testing was performed at Weeks 0, 6 and 12. Hematology parameters and pseudocholinesterase activity were measured at termination. All animals were necropsied and brain, heart, kidney, liver, lung, testis and thymus were weighed. Tissues from all control and high-dose animals were examined microscopically. In addition, a group of 16

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animals/sex/dose were included for neurobehavioral testing.

Results: Male mice were more sensitive than females to exposure to methyl bromide. Four/27 males in the 120 ppm group were sacrificed moribund during the study. Clinical signs observed at 120 ppm included severe curling and crossing of the hindlimbs and forelimb twitching. The study report indicated that these effects were dose and time related and that effects were more severe in males than females, but a summary of the clinical data was not provided. Mild neurobehavioral effects were sporadically observed and included increased latencies for startle response and hot plate activity in males at Week 6 and decreased latency for activity in females at Week 12. Body weight was affected at high dose in males (88% of controls) but not females.

Slightly decreased mean cell hemoglobin and cell volume (20% less than controls) and increased erythrocyte count (34% more than controls) were observed in males at 120 ppm and also to a lesser extent at 40 and 80 ppm. No clear treatment-related effects on hematologic parameters were observed in females (sporadic differences were observed at all dose levels), and pseudocholinesterase levels were not affected in either sex.

Organ weights were not affected and no treatment-related microscopic changes were observed at 120 ppm, including those that were killed moribund during the study.

13-Week Inhalation Exposure Study in Rats

Study Design: Ten F344/N rats/sex/dose were exposed by inhalation to methyl bromide vapor at concentrations of 0, 30, 60 or 120 ppm (0, 0.1163, 0.2325 or 0.4651 mg/l). Exposures were conducted for 6 hrs/day, 5 days/week over 13 weeks. Animals were observed twice daily for clinical signs and were weighed weekly. In addition, groups of 8 rats/sex/dose were included for neurobehavioral assessment at 0, 3, 6, 9 and 13 weeks. Hematology parameters and pseudocholinesterase were measured at termination. Necropsy was performed on all animals and adrenal glands, brain, heart, kidney, liver, lung, spleen and testis were weighed. Tissues from all control and high-dose animals were examined microscopically.

Results: No mortality occurred during the study. Daily observations did not reveal treatment-related clinical signs. Minor neurologic effects were occasionally observed in the neurobehavioral assessment at 120 ppm, including slight but statistically significant decreases in hindlimb grip strength, startle response amplitude, activity latency and increased novel side crossing frequency (males), and decreased forelimb grip strength, startle response amplitude and increased startle response latency. Mean body weight and body weight gain were decreased at 120 ppm in both males and females (87-88% of controls); a smaller but statistically significant

decrease (94% of controls) was also observed in females at 60 ppm. Statistically significant decreases in RBC parameters were observed in females but because of the small magnitude (4 - 9%) did not appear to be biologically significant.

Olfactory epithelial dysplasia and cyst formation were observed at 120 ppm in 7 - 9/10 males and females. In contrast, rats exposed to 0, 30 or 60 ppm methyl bromide vapor had low incidence of dysplasia (2/10, 3/10 or 2/9, males; 1/10, 1/10 or 4/10, females) and did not have cyst formation.

TARGET ORGAN STUDIES

[Note: Target organ studies were also published in a journal article: Eustis, S.L., Haber, S.B., Drew, R.T. and Yang, R.S.H. (1988) Fundam. and Applied Toxicol. 11: 594-610.]

6-Week Target Organ Study in Mice:

Study Design: Five B6C3F$_1$ mice/sex were exposed by inhalation to 0 ppm or 160 ppm (0 or 0.6201 mg/l) methyl bromide vapor for a total of 10 exposures, and 15 mice/sex to 160 ppm methyl bromide vapor for 30 exposures. Exposures were for 6 hrs/day, 5 days/week. It was predetermined that exposures would be terminated and all animals sacrificed if mortality reached 50% before scheduled sacrifice time. Animals were observed twice daily for clinical signs and mortality. Body weights were measured on the day before exposure, weekly thereafter and at termination. Complete necropsies were performed and brain, testes, thymus, spleen, heart, liver, kidneys, lung and nasal cavity from all animals were weighed and examined microscopically.

Results: Due to high mortality, exposures to 160 ppm were terminated and all surviving animals sacrificed after 10 exposures (males) or 8 exposures (females). Mortality was observed at 160 ppm in males (4/20 survivors at Day 10 termination) and females (10/20 survivors at Day 8 termination). Clinical signs included lethargy, red urine and neurologic effects including curling and crossing of the hindlimbs, forelimb twitching and tremors. The incidence of clinical signs was not included in the study report.

Summary tables of results taken from the published study are attached (hematology, organ weight and histopathology data). Mean body weights of surviving treated males and females were reduced (25% less than control weights). Statistically significant decreases in the weights of some organs (lung, heart, kidney, thymus, liver) were observed in both sexes and may have been related to decreased body weight. The most pronounced decreases occurred in liver, lung and heart (~25-30% less than controls) and thymus (~60% less than controls). Mean brain

weights were decreased by 5 - 7%.

Microscopic lesions were observed in the brain, kidney, testes, nasal epithelium and thymus. Neuronal necrosis was observed in the internal granular layer of the cerebellar folia with slightly greater severity and frequency in males. Very slight necrosis of the pyramidal neurons of the cerebral cortex was also observed (nuclear pyknosis, vacuolization of the perikaryon). Renal nephrosis was noted in all exposed animals; greater severity of some lesions in males may have been due to increased duration of exposures. Minimal testicular degeneration and nasal olfactory epithelium degeneration and atrophy were also seen in males (one female had olfactory epithelial degeneration). Degeneration of the myocardium was more pronounced in males than females. Adrenal gland in female mice showed decreased cellularity of the x-zone with smaller, hyperchromatic nuclei. Both the thymus and spleen showed lymphoid depletion, which was often severe in the thymus and may have accounted for the reduced thymic weight at termination.

Surviving female mice had pronounced depression of RBC and hemoglobin (35% less than controls) and increased WBC count (320% of controls). No hematologic effects were observed in males. Clinical chemistry and urinalysis parameters were stated to have been unaffected in both sexes; however no data was presented in the report. The clinical observation of red urine was apparently not confirmed in the urinalysis.

Conclusions: Based on the results of this study, the target organs of methyl bromide in mice are brain, heart, testis, olfactory epithelium and adrenal gland in females. Thymus and spleen were also affected (lymphoid depletion), possibly secondary to stress. Males appeared to be more sensitive than females, based on mortality rate and number of animals affected with most types of microscopic lesions, including cerebral cortical neuronal necrosis, cardiac degeneration, thrombi in lung and degeneration/atrophy of nasal cavity olfactory epithelium.

6-Week Target Organ Study in Rats:

Study Design: Five F344 rats/sex/dose were exposed by inhalation to 0 or 160 ppm (0 or 0.6201 mg/l) methyl bromide vapor for 3, 10 or 30 days' exposure. An extra group of 5 rats/sex/dose were included in the 30-day exposure group for hematology, clinical chemistry and urinalysis. Exposures were conducted daily for 6 hrs/day, 5 days/week. It was predetermined that animals were to be sacrificed if mortality reached 50% before scheduled sacrifice. Animals were observed twice daily for clinical signs and mortality and were weighed at one day pretreatment and then weekly until termination (the 3-day group was weighed only pretreatment and at termination). All animals were necropsied and adrenal glands, brain, testes, thymus, spleen, heart, liver, kidneys, lung and nasal cavity were examined microscopically. All of these organs except thymus and nasal cavity were weighed.

Results: Male rats showed high mortality and were sacrificed after 14 exposures (5/10 surviving animals. Female rats were sacrificed at 30 days (5/10 surviving animals). The study report indicated that neurologic effects similar to mice (curling and crossing of hindlimbs, forelimb twitching and tremors) were observed in treated rats. Data on the incidence of clinical effects were not included in the report. No treatment-related effects on clinical pathology parameters were observed.

Data tables from the published study report summarizing body and organ weights, hematology and histopathology data are attached. Mean body weights at sacrifice were decreased in males (32% less than controls) and females (18%). The report stated that there was indication of decreased body weight and some organ weights after 3 and 10 days' exposure, but data was not included in the report. Reduced lung, heart, spleen, brain, liver and testicular weights were observed in male and/or females. In males, the most pronounced decreases were observed in liver (45% less than controls), spleen (46%), testes (25%) and lung (27%). In females, liver weight was lower in treated animals (22%). Other decreases in organ weight were less pronounced (10 - 15%) and of uncertain biological significance.

Microscopic lesions were observed in the brain, kidneys, testes, nasal cavity, heart, adrenal gland, liver, thymus and spleen. Necrosis and neuronal loss were observed in cerebral cortex, hippocampus and thalamus and females were affected more often than males. In addition, neuronal loss and gliosis and minimal effects in the external pyramidal layer neurons (shrunken cells, pyknotic nuclei, pale cytoplasm) were observed in a few females. The study authors attributed the increased severity of lesions in females to the longer duration of exposure rather than sex differences. Kidneys were not affected except for one female, which had minimal nephrosis and sloughing of tubular epithelium in the renal cortex. Testicular degeneration and seminiferous tubule atrophy were observed in several rats. Moderate to marked degeneration of olfactory epithelium was observed in the ethmoturbinates and posterior dorsal nasal septum in animals killed after 3 exposures. After 10 or more exposures, degeneration appeared less severe but focal/multifocal loss of olfactory sensory cells was observed. Myocardial degeneration, single cell necrosis of the liver and minimal to mild cytoplasmic vacuolization of the adrenal gland were also observed. Thymic and splenic lymphoid depletion was observed and thymic atrophy were often severe.

Based on the results of this study, the major target organs of methyl bromide in rats are brain, liver, testes, heart, olfactory epithelium and possibly adrenal gland. In addition, lymphoid depletion of the thymus and spleen were observed and may have been secondary to stress. As with the mice, males tended to be more sensitive to methyl bromide, based on mortality rate and the number of animals affected by some of the microscopic lesions observed (in rats, necrosis of the cerebral cortex, hippocampus and thalamus).

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SPERM MORPHOLOGY AND VAGINAL CYTOLOGY STUDIES

Methods: Ten mice and ten rats/sex/dose were exposed by inhalation to 0, 10, 40, 80 or 120 ppm methyl bromide vapor for 13 weeks as described for the 13-week study, above. In males, body weight, right caudal weight, right epididymal weight, right testicular weight, sperm motility, sperm density and sperm head morphology were evaluated at the end of the exposure period. In the females, the total length of the estrous cycle and relative frequencies of the estrous stages were determined along with an analysis of vaginal cytology.

Results: In rats, vaginal morphology and estrous cycle were unaffected by exposure to methyl bromide. However, sperm motility was significantly decreased at 120 ppm (65% less than control activity). Sperm density was not affected.

In mice, vaginal cytology was not affected by exposure to methyl bromide. Estrous cycle was slightly lengthened (to 4.5 days from 4.0 days); however, the study author questioned the significance of this finding. Sperm density was significantly decreased (about 33% less than controls). Sperm mobility was not tested.

Taken from published 6-week target organ study

TABLE 2

SUMMARY OF BODY AND ORGAN WEIGHTS OF B6C3F1 MICE AND F344 RATS FOLLOWING REPEATED INHALATION EXPOSURE TO 160 ppm METHYL BROMIDE

	Male mice		Female mice	
Concentration (ppm):	0 $n = 20$	160 $n = 4$	0 $n = 20$	160 $n = 10$
Body weight (g)	27.3 ± 0.32	20.1 ± 1.48[c]	20.2 ± 0.25	16.53 ± 0.54[c]
Lung (g)	0.19 ± 0.00	0.14 ± 0.01[c]	0.17 ± 0.00	0.12 ± 0.01[c]
Heart (g)	0.17 ± 0.00	0.13 ± 0.01[b]	0.12 ± 0.00	0.09 ± 0.00[c]
Spleen (g)	0.08 ± 0.01	0.05 ± 0.02[a]	0.08 ± 0.00	0.10 ± 0.01
Right kidney (g)	0.29 ± 0.01	0.24 ± 0.02[a]	0.19 ± 0.00	0.17 ± 0.01
Thymus (g)	0.05 ± 0.00	0.02 ± 0.00[a,c]	0.07 ± 0.01	0.03 ± 0.00[c]
Brain (g)	0.45 ± 0.01	0.43 ± 0.01[a]	0.45 ± 0.01	0.42 ± 0.01[c]
Liver (g)	1.57 ± 0.04	1.18 ± 0.15[c]	1.22 ± 0.02	0.95 ± 0.05[c]
Right testes (g)	0.10 ± 0.01	0.10 ± 0.00		

	Male rats		Female rats	
Concentration (ppm):	0 $n = 10$	160 $n = 5$	0 $n = 10$	160 $n = 5$
Body weight (g)	164.2 ± 3.0	112.0 ± 10.3[c]	150.5 ± 3.4	123.6 ± 2.8[c]
Lung (g)	1.01 ± 0.03	0.73 ± 0.34[c]	0.87 ± 0.02	0.78 ± 0.03[a]
Heart (g)	0.66 ± 0.02	0.58 ± 0.02[a]	0.57 ± 0.01	0.54 ± 0.01
Spleen (g)	0.41 ± 0.01	0.22 ± 0.04[c]	0.39 ± 0.01	0.38 ± 0.01
Right kidney (g)	0.77 ± 0.02	0.59 ± 0.03[c]	0.65 ± 0.02	0.55 ± 0.01[a]
Adrenals (g)	0.05 ± 0.00	0.05 ± 0.01	0.05 ± 0.00	0.06 ± 0.00
Brain (g)	1.66 ± 0.02	1.56 ± 0.02[a]	1.68 ± 0.02	1.52 ± 0.03[c]
Liver (g)	6.95 ± 0.19	3.77 ± 0.36[c]	6.00 ± 0.21	4.65 ± 0.33[b]
Right testes (g)	1.06 ± 0.03	0.76 ± 0.15[a]		

Note. All values expressed as $\bar{X}$ ± SE. Male mice killed after 10 exposure days; female mice killed after 8 exposure days. Male rats killed after 14 exposure days; female rats killed after 30 exposure days.

[a] $0.01 < p < 0.05$.
[b] $0.001 < p < 0.01$.
[c] $p < 0.001$.
[d] $n = 3$.

TABLE 3

SUMMARY OF HEMATOLOGICAL EVALUATION OF B6C3F1 MICE FOLLOWING REPEATED INHALATION EXPOSURE TO 160 ppm METHYL BROMIDE

	Male mice		Female mice	
Concentration (ppm):	0 (n)	160 (n)	0 (n)	160 (n)
RBC (10^6/mm^3)	8.86 ± 0.19 (19)	8.58 ± 0.18 (3)	10.00 ± 0.07 (20)	6.64 ± 0.26[c] (10)
WBC (10^3/mm^3)	9.54 ± 0.47 (19)	9.83 ± 2.04 (3)	7.90 ± 0.31 (20)	25.52 ± 5.22[c] (10)
HgB (g %)	16.72 ± 0.29 (19)	16.57 ± 0.32 (3)	16.42 ± 0.10 (19)	11.30 ± 0.59[c] (9)
HCT (%)	50.18 ± 0.74 (20)	51.31 ± 0.95 (4)	50.44 ± 0.31 (20)	38.88 ± 2.31[c] (10)
MCV	56.66 ± 0.55 (19)	60.45 ± 1.83[a] (3)	50.50 ± 0.43 (20)	58.54 ± 2.72[c] (10)
MCH	18.91 ± 0.14 (19)	19.30 ± 0.13 (3)	16.45 ± 0.10 (19)	17.12 ± 0.16[c] (9)
MCHC	33.38 ± 0.16 (19)	31.98 ± 0.80[a] (3)	32.64 ± 0.19 (19)	29.71 ± 1.19[b] (9)

Note. All values expressed as $\bar{X}$ ± SE. Males killed after 10 exposure days; females killed after 8 exposure days.

[a] $0.01 < p < 0.05$.
[b] $0.001 < p < 0.01$.
[c] $p < 0.001$.

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Taken from 6-week target organ study (published report).

TABLE 5

SUMMARY INCIDENCES OF EXPOSURE-RELATED HISTOPATHOLOGICAL LESIONS IN MICE

	Male mice		Female mice	
	0 ppm	160 ppm	0 ppm	160 ppm
Adrenal gland (No. examined)	(20)	(20)	(20)	(18)
x-Zone–Atrophy				16
Brain (No. examined)	(20)	(20)	(20)	(20)
Cerebral cortex–Neuronal necrosis		11		
Cerebellum–Neuronal necrosis		12		10
Hemorrhage		1		
Testes (No. examined)	(20)	(20)		
Atrophy	2	2		
Degeneration	1	15		
Necrosis		1		
Thymus (No. examined)	(20)	(4)	(19)	(7)
Atrophy		4	1	6
Spleen (No. examined)	(20)	(20)	(20)	(20)
Lymphoid depletion	1	17		17
Hematopoiesis	2	7		7
Red pulp cellular depletion				8
Heart (No. examined)	(20)	(20)	(20)	(20)
Degeneration		14		2
Kidney (No. examined)	(20)	(20)	(20)	(20)
Nephrosis		20		19
Lung (No. examined)	(20)	(19)	(20)	(19)
Congestion		4		
Hemorrhage		4		
Thrombi		8	1	5
Nasal cavity (No. examined)	(20)	(20)	(20)	(20)
Olfactory epithelium degeneration		16		1
Olfactory epithelium atrophy		12		

Note. Male mice were killed after 10 exposure days and female mice after 8 exposure days because the mortality rate exceeded 50%.

Taken from published 6-week target organ study

TABLE 4

SUMMARY INCIDENCES OF EXPOSURE-RELATED HISTOPATHOLOGICAL LESIONS IN RATS

	Male rats						Female rats					
	0 ppm			160 ppm			0 ppm			160 ppm		
Adrenal gland (No. examined)	(5)	(5)	(10)	(5)	(5)	(10)	(5)	(5)	(10)	(5)	(5)	(10)
Cortex–Cytoplasmic Vacuolation					4	10					5	9
Brain (No. examined)	(5)	(5)	(10)	(5)	(5)	(10)	(5)	(5)	(10)	(5)	(5)	(10)
Cerebral cortex–Neuronal necrosis						5						10
Cerebral cortex–Gliosis						1						2
Hippocampus–Neuronal necrosis						2						4
Hippocampus–Gliosis												4
Thalamus–Neuronal necrosis												2
Thalamus–Gliosis												
Cerebellum.–Mineralization												
Testes (No. examined)	(5)	(5)	(10)	(5)	(5)	(10)						
Degeneration	1					3						
Atrophy						2						
Thymus (No. examined)	(5)	(5)	(10)	(5)	(5)	(9)	(5)	(5)	(10)	(5)	(5)	(10)
Necrosis						4						3
Atrophy						5						4
Spleen (No. examined)	(5)	(5)	(10)	(5)	(5)	(10)	(5)	(5)	(10)	(5)	(5)	(10)
Lymphoid depletion						2						4
Hemosiderosis												7
Heart (No. examined)	(5)	(5)	(10)	(5)	(5)	(10)	(5)	(5)	(10)	(5)	(5)	(10)
Degeneration	1	1	7	3	5	10	2	1	9	5	5	10
Liver (No. examined)	(5)	(5)	(10)	(5)	(5)	(10)	(5)	(5)	(10)	(5)	(5)	(10)
Inflammation, subacute focal		2	1			3			2	1	1	6
Necrosis						6						2
Nasal cavity (No. examined)	(5)	(5)	(10)	(5)	(5)	(10)	(5)	(5)	(10)	(5)	(5)	(10)
Olfactory epithelium degeneration				5	3	7				5	5	9
Olfactory epithelium atrophy					5	10					5	10

Note. Male rats were killed after 14 exposure days because the mortality rate exceeded 50%.

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[Methyl Bromide, technical]

Mouse Carcinogenicity Study 83-2

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Linnea T. Hansen, Date 9/8/94

, Date 9/21/94

DATA EVALUATION REPORT

STUDY TYPE: Chronic Inhalation Toxicity/Carcinogenicity - Mouse (83-1a, 83-2b)

TOX. CHEM. NO: 555

P.C.CODE.: 053201

MRID NO.: 425041-01

TEST MATERIAL: Methyl bromide

SYNONYMS: Bromomethane

STUDY NUMBER: Laboratory Project Identification NTP TR 385

SPONSOR: Methyl Bromide Industry Panel

TESTING FACILITY: National Toxicology Program, Brookhaven National Laboratories, Integrated Laboratory Systems, Experimental Pathology Laboratories and Biotechnology Services, Inc.

TITLE OF REPORT: Methyl Bromide: Toxicology and Carcinogenesis Studies of Methyl Bromide in B6C3F, Mice (Inhalation Studies)

AUTHOR: S.L. Eustis, D.V.M.

REPORT ISSUED: March, 1992

EXECUTIVE SUMMARY: In a 2-year chronic toxicity/carcinogenicity study, 50 B6C3F1 mice/sex/dose were exposed for 102 weeks to methyl bromide vapor at atmospheric levels of 0, 10, 33 or 100 ppm (0, 0.03876, 0.1279 or 0.3876 mg/l). In addition, groups of 10 animals/sex/dose were exposed for interim sacrifice at 6 months and at 15 months, and groups of 16 animals/sex/dose for neurobehavioral assessment at 3 month intervals. Exposures were conducted 5 days/week for 6 hrs/day. Exposure of all high dose animals was terminated after 20 weeks due to excessive mortality in males and males from the interim sacrifice and neurobehavioral groups were incorporated into the main group to ensure adequate survivors.

At 100 ppm, survival was markedly decreased in males (23% vs. 82%, controls, at termination).

Clinical signs in males and females included abnormal posture, tremors, ataxia, limb paralysis and emaciation. Some neurobehavioral effects were also observed (including increased startle response, decreased general activity). Decreased mean body weight gain was also observed (-56% less than controls, males and -41% less than controls, females). Microscopic lesions included degeneration of the cerebellum (44%, males; 18%, females vs. 0% in controls), cerebral degeneration (16%, males, 3%, females vs. 0% in controls), chronic cardiomyopathy (54%, males, 58%, females vs. 8% and 2%, male and female controls), cardiac degeneration (45%, males and 12%, females vs. 2%, female controls), sternal dysplasia (15 - 20% vs. 0% in controls) and olfactory epithelium metaplasia or necrosis (2 - 9% vs. 0% in controls). **The LOEL for systemic toxicity in this study is 100 ppm (0.3876 mg/l), based on mortality (males), neurological signs, decreased body weight/weight gain and microscopic lesions in the brain, heart, sternum and olfactory epithelium. The NOEL for systemic toxicity is 33 ppm (0.1279 mg/l).**

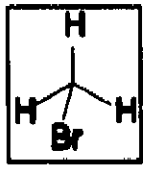
There was no evidence of carcinogenic potential of methyl bromide. Although excessive toxicity occurred at 100 ppm, the dosing is considered adequate because (1) methyl bromide has a steep dose-time dependent mortality curve and (2) at 33 ppm, marginally lower mean body weight gain in females (-12%) and sporadic incidence of abnormal posture late in the study (1 male, 3 females) may represent a threshold NOEL.

This study is Core-minimum and satisfies the guideline requirement for a carcinogenicity study in mice (83-2b), despite some problems with the dose selection (see Discussion/Conclusions in DER). The study does not satisfy the guideline requirement for a chronic toxicity study (83-1a) in rodents due to study deficiencies including no clinical chemistry data, no urinalysis and no reporting of gross findings. However, although it was submitted for both 83-1a and 83-2b, a rodent chronic inhalation toxicity study is not required for methyl bromide.

Special Review Criteria (40 CFR 154.7) None

A. MATERIALS:

1. Test Material: Methyl Bromide, technical
   Description: colorless, odorless gas
   Lot/Batch #: E21-1012-00 (Matheson Gas Products, Joliet, IL)
   Purity: 99.8% a.i.
   Stability of compound: stable stored under pressure at room temperature
   CAS #: 74-83-9
   Structure



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2. Test animals: Species: Mouse
Strain: $B6C3F_1$
Age and weight at study initiation: 6 weeks/11.0 - 26.5, males; 9.7 - 19.5, females.
Source: Frederick Cancer Research Facility, Frederick, MD
Housing: Individually, stainless steel wire cages
Environmental conditions: Temperature: 65 - 82°F
Humidity: 11 - 85%
Air changes: 15/hr
Photoperiod: 12 hr light/12 hr dark
Acclimation period: 2 weeks including 8 day quarantine

B. STUDY DESIGN:

1. Animal assignment

Animals were assigned randomly to the test groups as shown in Table 1.

TABLE 1: STUDY DESIGN

Test Group	Exposure (ppm)	Main Study 24 months		Interim Sac. 6 months		Interim Sac. 15 months		Neurobehav. tests Every 3 mos.	
		male	female	male	female	male	female	male	female
1 Control	0	50	50	10	10	10	10	16	16
2 Low (LDT)	10	50	50	10	10	10	10	16	16
3 Mid (MDT)	33	50	50	10	10	10	10	16	16
4 High (HDT)	100	50	50	10	10	10	10	16	16

Changes to study design, high dose animals: Due to unexpectedly high mortality in males at 100 ppm, exposure of high dose males and females to methyl bromide vapor was discontinued after 20 weeks (animals were exposed only to air for the duration of the study). Males from the 100 ppm interim sacrifice and the neurobehavioral testing groups were reassigned to the main (2-year) 100 ppm group to ensure adequate numbers of surviving animals at termination. Neurobehavioral testing of males at 100 ppm was not performed after 3 months. The 10 females at 100 ppm intended for the 6-month sacrifice were reassigned to the main group.

Dose selection rational: Atmospheric chamber concentrations of methyl bromide in this study were selected based on results of a 13-week inhalation study in mice conducted by the same laboratory. Mice were exposed to 0, 10, 20, 40, 80 or 120 ppm methyl bromide vapor for 6 hrs/day, 5 days/week. Signs of toxicity were observed at 120 ppm and included decreased body weight (males) and clinical signs indicative of neurotoxicity. Details of this study, as well as other toxicity studies conducted in the NTP evaluation of methyl bromide (14-day mouse inhalation study, 13-week rat inhalation study, 6-week rat and mouse target organ toxicity studies, vaginal cytology study and sperm morphology study) are summarized in greater detail in the Appendix of the cover memorandum for this study.

2. Test Atmosphere Generation and Analysis

Exposure Chambers and Generation of Test Atmosphere: Mice were exposed to methyl bromide vapor or chamber air into 1.4 m^3 stainless steel and glass or lucite exposure chambers. Vapor was delivered from compressed gas cylinders via a shrouded delivery tube to a distribution plenum. Rotameters were used to control gas flow to each chamber. Chambers reached 90% of target concentration within 20 minutes of initiation of delivery. Chamber atmospheres were exhausted from the bottoms of the chambers through charcoal filters.

Chamber concentrations of methyl bromide were measured using a MIRAN 80 infrared spectrophotometer. Air samples from each chamber were removed hourly for analysis (each sample collected for 10 minutes) during exposures. Uniformity of vapor distribution in each chamber was measured from 8 ports. The study report did not indicate whether oxygen concentration was measured during the study.

Results - The 10 ppm chamber concentration measurements varied from 90 - 120% of target concentration during the first week of exposure; thereafter, all but a few measurements were within 110% of target. At 33 and 100 ppm, all measurements were within 10% of target. Spatial variation of the distribution of methyl bromide vapor in each chamber did not exceed 4.2%. The occasional variations observed in this study at 10 ppm were not likely to have affected the results of this study.

3. Animals received food (NIH-07 Rat and Mouse Ration, Zeigler Bros., Inc.) and tap water ad libitum, except during inhalation exposures.

4. Statistics - Survival probability was estimated using the product-limit procedure of Kaplan and Meir. The method of Cox for testing groups for equality was used to determine dose-related effects on survival and Tarone's life table test was used to analyze dose-related trends of survival (two-sided P values).

Incidence of tumors that were considered incidental to death or not rapidly lethal was analyzed by logistic regression analysis. Intercurrent mortality was adjusted using prevalence analysis of Dinse and Lagakos, and Dinse and Hazeman. Rapidly lethal tumors were analyzed using the life table test of Cox or Tarone. The overall proportion of tumor-bearing animals were analyzed using the Fisher exact test and Cochran-Armitage trend test.

Continuous variables (organ weight and hematologic data) were analyzed using either Dunn's or Shirley's multiple comparison test and Jonckheere's test was used to determine significance of the dose response trends and which comparison test was appropriate. Body weight data and incidence of clinical signs were not analyzed statistically.

Statistical significance was determined by pair-wise comparisons to controls and a dose-response

trend test. Continuity-corrected tests were used for analysis of tumor incidence (one-sided p values). These methods were also used to analyze some nonneoplastic microscopic lesions.

5. Signed and dated quality assurance and GLP statements were not present. However, in the study report it was stated that the study was conducted in compliance with FDA GLP regulations (21 CFR Part 58) and that an independent quality assurance contractor audited the study records.

C. METHODS AND RESULTS:

1. Observations:

Animals were inspected twice daily (once on weekends for the first year) for signs of toxicity and mortality.

Results - Survival is shown below in Table 2:

TABLE 2: SURVIVAL AT SELECTED TIME POINTS DURING THE STUDY[1,2]

Week of study	0 ppm	10 ppm	33 ppm	100 ppm[3]
Males:				
10	86[4]	85	85	85
20	86	85	85	59
30	70	70	71	39
50	69	70	71	37
78	53	53	55	26
100	50	49	46	18
Term. sac.	48	45	46	16
% survival at termination	82%	74%	80%	23%
Females:				
10	84	86	86	85
20	84	86	86	79
30	65	71	72	72
50	68	71	72	70
78	50	54	58	54
100	46	50	53	46
Term. sac.	43	48	51	46
% survival at termination	71%	82%	90%	65%

1 Data taken from Tables 7 and 8 of study report
2 Scheduled interim sacrifices were performed between weeks 26 - 30 and between weeks 66 and 70, except no interim sacrifices were performed on high-dose males or at 6 months in high-dose females. See study design for number of animals sacrificed at each time.
3 Exposure at 100 ppm terminated after 20 weeks because of high mortality
4 Values indicate number of animals surviving at each indicated time

Survival in males was markedly decreased at 100 ppm. Survival was comparable to controls among all treatment groups until Week 12, after which survival at 100 ppm began to suddenly decrease: Between Weeks 12 and 20 survival was reduced from 95% to 67%; most of these deaths were moribund sacrifices. Exposure of high dose males and females to methyl bromide was completely terminated after 20 weeks due to the steep time-dose response for mortality. Despite termination of exposure, high-dose male survival continued to decrease throughout the study compared to all other groups. Survival was not affected in the 10 or 33 ppm male groups or in females at any exposure level.

Clinical signs observed during the study are summarized below in Table 3:

TABLE 3: SELECTED CLINICAL FINDINGS[1]

Clinical Finding	0 ppm	10 ppm	33 ppm	100 ppm
MALES: (N, study start)	(86)	(85)	(86)	(86)
Abnormal posture	0[2]	0	1	41
Tremors	0	0	0	50
Ataxia	0	0	0	20
Limb paralysis	0	0	0	23
Dyspnea	1	2	0	1
Tachypnea	1	0	1	3
Emaciation	1	0	0	19
Hypoactivity	1	2	1	3
Head tilt/circling	0	1	0	0
None of above ("normal")	85[3]	81	83	18
FEMALES: (N, study start)	(87)	(86)	(86)	(86)
Abnormal posture	0	0	3	30
Tremors	0	0	0	14
Ataxia	0	0	0	6
Limb paralysis	0	0	0	8
Dyspnea	1	1	0	4
Tachypnea	0	1	2	2
Emaciation	0	1	0	5
Hypoactivity	2	0	2	4
Head tilt/circling	2	2	0	0
None of above ("normal")	82	80	80	43

1 Data extracted from Attachment VII; Individual Animal History; pp. 312-953. Includes interim sacrifice and neurobehavioral group animals.
2 Values indicate the number of animals affected and do not reflect frequency or severity of observation.
3 Animals that did not have any of the listed symptoms may have had alopecia, swellings, tissue masses, wounds or other observations unrelated to treatment.

At 100 ppm, neurological signs including tremors, abnormal posture (due to curvature of the spine), ataxia and limb paralysis were observed in males and females. Males tended to be more severely affected than females and generally had earlier onset of symptoms (earliest onset of symptom 65 days vs. 114 days for females; more than 50% of males with symptoms had onset by Day 150, vs. 25% in females). Symptoms in the

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high-dose animals were generally observed throughout the study until terminal or unscheduled sacrifice, although frequently signs were observed intermittently following the initial observation. Emaciation was frequently observed among males and in some females at 100 ppm and correlated with reduced body weight gain. A small number of control, low- and mid-dose animals were affected with some of these symptoms (abnormal posture, hypoactivity, dyspnea). However, these symptoms were observed at low incidence and generally not until the last several months of the study (usually after Day 600), and were considered of questionable relationship to treatment. Alopecia and swelling of limbs, tail or other regions were frequently observed among all groups (data not shown).

2. Body weight

Animals were weighed weekly for the first 13 weeks, every 4 weeks for the next 18 months and then every 2 weeks until termination.

Results - Mean body weight data from selected intervals in the study are shown below in Table 3:

TABLE 2: REPRESENTATIVE MEAN BODY WEIGHTS AND TOTAL BODY WEIGHT GAIN, GRAMS[1,2]

INTERVAL	0 PPM	10 PPM	33 PPM	100 PPM
MALES:				
WEEK 1	22.9	22.8	21.6	21.3
10	30.9	31.1	30.8	29.9
22	37.7	36.4	37.6	29.0
50	46.7	45.8	46.3	30.5
78	47.5	47.6	46.7	30.5
100	47.7	46.5	46.4	32.0
MEAN TOTAL GAIN (WEEKS 1-100):	24.3	23.7	24.8	10.7 (56%)
FEMALES:				
WEEK 1	18.1	19.4	18.3	18.6
10	24.8	24.4	23.8	23.7
22	30.0	28.6	28.4	24.7
50	37.7	38.2	36.4	32.8
78	43.0	42.1	39.3	33.6
100	44.5	44.3	40.9	32.5
MEAN TOTAL GAIN (WEEKS 1-100):	26.4	26.1	23.3 (12%)	15.5 (41%)

1 Data taken from Tables 3 and 5 of study report. Body weight data not analyzed statistically.
2 Numbers in parentheses represent % decrease in total gain less than controls

Significant treatment-related decreases in mean body weight were observed in males and females (33% and 27% less than controls, respectively) at 100 ppm. Pronounced decreases in mean body weight were observed by Week 11 and persisted until termination. Body weight gain was not calculated by the study author; at 10, 30 and 100 ppm, mean body weight gains in males and

females were 56% and 41% less than controls, respectively. Marginally decreased body weight gain (12% less than controls) was observed in females at Week 100 at 33 ppm. Since food consumption was not measured, it could not be determined whether animals at high dose ate less, eg. due to neurotoxicity effects, or whether weight gain was reduced due to another mechanism.

3. Food consumption: Not evaluated in this study since exposure route was via inhalation.

4. Ophthalmoscopic examination: Not conducted in this study.

5. Blood was collected at 6, 15 and 24 months for hematology analysis from all surviving animals. The CHECKED (X) parameters were examined.

a. Hematology

X		X	
X	Hematocrit (HCT)*	X	Leukocyte differential count*
X	Hemoglobin (HGB)*	X	Mean corpuscular HGB (MCH)
X	Leukocyte count (WBC)*	X	Mean corpusc. HGB conc.(MCHC)
X	Erythrocyte count (RBC)*	X	Mean corpusc. volume (MCV)
X	Platelet count*		Reticulocyte count
	Blood clotting measurements		
	(Thromboplastin time)		
	(Clotting time)		
	(Prothrombin time)		

* Required for subchronic and chronic studies

Results - No treatment-related effects on hematology parameters were observed in this study.

b. Clinical Chemistry: Not evaluated in this study

6. Urinalysis: Not performed in this study

7. Neurobehavioral Testing

Satellite groups of 16 animals/sex/dose were scheduled for neurobehavioral assessment at 3 month intervals. Startle response, locomotor activity, grip strength, hindlimb footsplay, hot plate and activity latency and novel side time and novel side crossing were evaluated. Males exposed to 100 ppm were only evaluated at 0 and 3 months; survivors of the satellite neurobehavioral testing group were then incorporated into the main study group due to unexpectedly high mortality. Details on the testing methods and measurement devices (eg. motor activity) were not included in the study report.

Results - Selected neurobehavioral parameters are shown in Table 4:

TABLE 4: SELECTED NEUROBEHAVIORAL PARAMETERS[1]

		0 ppm	10 ppm	33 ppm	100 ppm
Males - 3 mos.					
Startle response latency, msec		395	358	332	267**
Startle response, amplit.,		190	227	224*	307**
Activity latency, sec		18.3	17.6	26.8	86.0**
Locomotor activity		189	178	174	112**
Novel side time, sec		107.3	116.1	111.7	59.9*
Novel side crossing, frequency		7.8	7.2	6.9	2.6**
Hot plate latency, sec		9.5	10.3	9.6	15.5*
Hindlimb grip strength, g		57.3	52.9	54.6	74.5*
Females -					
Startle resp. latency	3 mos	373	373	365	230**
	12 mos	357	353	388	300
	24 mos	439	347	365	259*
Startle resp. amplit.	3 mos	201	199	210	333**
	12 mos	210	209	189	276
	24 mos	149	190	207	283
Activity latency	3 mos	29.7	16.8	23.7	22.5
	12 mos	9.2	12.4	18.8	28.8
	24 mos	11.8	25.1	8.2	18.3
Locomotor activity	3 mos	185	187	187	173
	12 mos	166	140**	152*	125**
	24 mos	129	125	150	117
Novel side time	3 mos	100.5	115.3	112.4	119.3**
	12 mos	124.4	128.6	120.0	127.8
	24 mos	140.0	136.0	110.0	115.3
Novel side crossing	3 mos	7.3	7.6**	8.2	7.3
	12 mos	6.2	3.6	6.0	2.6**
	24 mos	3.7	3.4	5.5	4.5
Hot plate latency	3 mos	7.9	8.1	8.1	11.0*
	12 mos	9.1	8.1	7.9	7.7
	24 mos	8.0	7.5	6.2	8.3

1 Data taken from Table B3 of the study report
* $p \leq 0.05$ ** $p \leq 0.01$

No treatment-related effects were observed at 10 or 33 ppm in either sex. At 3 months, male mice exposed to 100 ppm had statistically significant reductions in novel side time (-44% of controls), reduced crossing frequency (-67%) and locomotor activity (-41%). Latencies for activity and hot plate were increased (+470% and +163% of controls, respectively); startle

response was enhanced (increased magnitude and decreased response latency). A statistically significant increase was also observed in hindlimb grip strength (+25%). However, TB-I notes that the small number of animals examined in both male and female control groups at 24 months (6 vs. 8 - 16 at other doses) made comparisons difficult.

Female mice showed similar effects at 100 ppm, except for novel side time, although statistically significant changes in many affected parameters did not persist throughout the study. The magnitude of effect was comparable to males for novel side crossing, hot plate latency, activity latency and startle response but was less pronounced for locomotor activity (-25% of controls). Statistically significant effects were usually observed at the 3 and 12 month intervals except for startle response effects, suggesting that some recovery or a compensatory mechanism may have occurred with time and cessation of exposure. A similar tendency could not be established in males since only one evaluation was performed.

8. Sacrifice and Pathology

All animals that died and that were sacrificed on schedule were subject to microscopic pathological examination of the CHECKED (X) tissues listed below. The (XX) organs, in addition, were weighed.

X		X		X	
Digestive system		Cardiovasc./Hemat.		Neurologic	
	Tongue		Aorta*	XX	Brain*,
X	Salivary glands*	X	Heart*	X	Periph. nerve*
X	Esophagus*	X	Bone marrow*	X	Spinal cord (3 levels)*
X	Stomach*	X	Lymph nodes*	X	Pituitary*
X	Duodenum*	XX	Spleen		Eyes (optic n.)*
X	Jejunum*	XX	Thymus*	Glandular	
X	Ileum*	Urogenital		XX	Adrenal gland*
X	Cecum*	XX	Kidneys*+		Lacrimal gland
X	Colon*	X	Urinary bladder*	X	Mammary gland*
X	Rectum*	XX	Testes*·	X	Parathyroids*··
XX	Liver *·	X	Epididymides	X	Thyroids*··
X	Gall bladder*	X	Prostate	Other	
X	Pancreas*	X	Seminal vesicle	X	Bone*
Respiratory		X	Ovaries*·		Skeletal muscle*
X	Trachea*	X	Uterus*	X	Skin*
XX	Lung*			X	All gross lesions and masses*
X	Nose				
	Pharynx				
X	Larynx				

* Required for subchronic and chronic studies.
· Organ weight required in subchronic and chronic studies.
·· Organ weight required for non-rodent studies.

Results - a. Organ weight - Absolute and relative organ weights are shown below in

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[Methyl Bromide, technical] Mouse Carcinogenicity Study 83-2

Table 5:

TABLE 5: SELECTED ABSOLUTE (G) AND RELATIVE (MG ORGAN WT/G BODY WT) ORGAN WEIGHTS AT TERMINATION[1]

	0 PPM		10 PPM		33 PPM		100 PPM	
ORGAN	ABS	REL	ABS	REL	ABS	REL	ABS	REL
MALES: N =	(40)	(40)	(37)	(37)	(40)	(40)	(16)	(16)
Brain	0.47	10.7	0.48	10.6	0.48	10.9	0.46	15.5**
Heart	0.26	5.9	0.25	5.6	0.25	5.6	0.21**	6.8*
Kidney	0.43	9.7	0.45	9.9	0.43	9.8	0.35**	11.4**
Liver	2.87	67.9	2.94	66.6	2.72	64.2	1.85**	62.6
Lung	0.28	6.3	0.29	6.5	0.29	6.8	0.22**	7.2**
Spleen	0.12	2.9	0.24	5.2	0.17	4.3	0.09**	3.0
Testes	0.12	2.7	0.09	2.0	0.12	2.6	0.11	3.5**
Thymus	0.06	1.2	0.09	1.9	0.06	1.3	0.02**	0.07
FEMALES: N =	(36)	(36)	(41)	(41)	(45)	(45)	(40)	(40)
Brain	0.49	11.5	0.49	12.4	0.49	12.3	0.49	15.6**
Heart	0.21	4.9	0.22	5.5	0.21	5.3	0.21	6.8**
Kidney	0.31	7.0	0.31	7.9	0.30	7.5	0.30	9.0**
Liver	1.96	45.2	2.20	56.7	2.10	51.7	2.10	55.6**
Lung	0.26	6.0	0.31	8.1	0.27	6.9	0.27	7.8**
Spleen	0.26	6.2	0.23	5.8	0.33	8.1	0.33	5.2
Thymus	0.07	1.4	0.05	1.2*	0.05**	1.1**	0.05**	1.0**

1 Data taken from Tables C9 and C10 of Study Report
* $p < 0.05$

Slight but statistically significant increases in relative weights of numerous organs were observed in males and females. TB-I agreed with the study author that this was probably related to reduced body weight. The only effect that TB-I considered possibly related to treatment was reduced thymus weight in males (-41%) and females (-29%). No significant effects were observed at interim sacrifice.

b. Gross pathology - No data on treatment-related gross lesions were included in the study report. The study report indicated that all animals were examined at necropsy but did not

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report gross lesions. Pathology was identified microscopically.

c. Microscopic pathology -

1) Non-neoplastic - Selected nonneoplastic microscopic lesions are shown in Table 6:

TABLE 6: INCIDENCE OF SELECTED NONNEOPLASTIC MICROSCOPIC LESIONS[1,2]

OBSERVATION	DOSE IN DIET, PPM 0	10	33	100
MALES: N = (unless otherwise noted)	50	50	50	70
Brain				
Cerebral degeneration	0	0	0	11 (16)-
Cerebellar degeneration	0	0	0	31 (44)-
Heart				
Degeneration	0	0	0	32 (46)-
Chronic Cardiomyopathy	4 (8)	7 (14)	10 (20)	24 (54)-
Bone, sternum metaplasia	0	0	3 (6)	14 (20)-
Olfactory Epithelium				
Metaplasia	0	0	1 (2)	2/69 (3)
Necrosis	0	0	0	6/69 (9)
Thymus				
Atrophy	1/41 (2)	1/36 (3)	1/41 (2)	11/42 (21)
Necrosis				
Spleen, atrophy	0	2 (4)	0	10 (14)
Liver, hepatocyte vacuolization	7 (14)	8 (16)	7 (14)	18 (26)
FEMALES				
Brain				
Cerebral degeneration	0	0	0	2 (3)
Cerebellar degeneration	0	0	0	11 (18)-
Heart				
Degeneration	1 (2)	0	0	7/59 (12)
Chronic cardiomyopathy	2 (4)	4 (8)	2 (4)	34/59 (58)-
Bone, sternum dysplasia	0	2 (4)	2 (4)	9 (15)-
Olfactory epithelium				
Metaplasia	0	0	0	5 (8)
Necrosis	0	0	0	1 (2)
Thymus, atrophy	1/45 (2)	1/40 (3)	0	4/51 (7)
Spleen, atrophy	0	1 (2)	1 (2)	4/60 (7)
Liver, hepatocyte vacuolization	0	0	2 (6)	3/60 (5)

1 Data taken from Tables 11 of study report.
2 Numbers in parentheses indicate percent incidence
* $p < 0.05$ ** $p \leq 0.01$ *** $p \leq 0.001$

Similar types of microscopic lesions were observed among males and females at 100 ppm but not at lower exposures. Brain, heart, olfactory epithelium and bone (sternum) were the primary target organs. Many of these lesions persisted in survivors despite termination of exposure after Week 20.

Pathologic lesions in the cerebellum were characterized by focal or diffuse nuclear pyknosis of the internal granular layer cells. In the cerebrum, focal cortical neuronal necrosis was observed along with neuropil edema, congestion and gliosis (mild). It was noted by the study author that brain degeneration lesions were more common in the animals with early mortality.

The myocardial degeneration is considered an acute change and was described as myofiber sarcoplasmic hyalinization and/or vacuolization and variable nuclear size, with mild interstitial hypercellularity. This also was observed almost exclusively in the animals that died early in the study (before Day 200). Chronic cardiomyopathy was described as myofiber atrophy, fibrosis and focal or diffuse mononuclear cellular infiltration, and was observed in the animals that survived or died after 18 months.

The small but dose-related incidence of sternal dysplasia was characterized by sternal distortion with ventral displacement of the manubrium into the thoracic inlet. The manubrium showed ventral or ventrolateral deviation and subluxation of other sternebrae, with lipping of the sternebral articular surfaces due to protruding mature cartilage and bone. The lesion was primarily observed in surviving animals rather than early deaths.

Olfactory epithelial necrosis was focal and observed in the early death animals (death before Day 138). Metaplasia, in contrast, was observed primarily among survivors and was described as focal replacement of olfactory epithelium with ciliated columnar epithelial cells.

Increases incidence of other lesions, considered secondary to stress, were observed in males and females, with higher incidence for most in males. These included thymic and splenic atrophy and vacuolization of hepatocytes were observed at 100 ppm compared to controls. Necrosis of the thymus was also seen in some high-dose males.

2) Neoplastic - Selected common neoplastic lesions observed in this study are shown below in Table 7:

TABLE 7: SELECTED NEOPLASTIC LESIONS[1]

Sex/Lesion	0 ppm	10 ppm	33 ppm	100 ppm
MALE:	N = 50	N = 50	N = 51	N = 70
Liver				
Hepatocellular adenoma	17 (34)[2]	19 (38)	17 (33)	7 (10)**
Hepatocellular carcinoma	14 (28)	16 (32)	10 (20)	4 (6)**
Lung				
Alv./bronchio. adenoma	12 (24)	8 (16)	10 (20)	4 (6)*
Alv./bronchio. carcinoma	2 (4)	8 (16)*	5 (10)	1 (1)
Malignant lymphoma	2 (4)	2 (4)	6 (12)	2 (3)
FEMALE:	N = 51	N = 50	N = 50	N = 62
Liver				
Hepatocellular adenoma	6 (12)	7 (14)	7 (14)	5 (8)
Hepatocellular carcinoma	4 (8)	4 (8)	2 (4)	1 (2)
Lung				
Alv./bronchio. adenoma	3 (6)	2 (4)	0	7 (11)
Alv./bronchio. carcinoma	4 (8)	4 (8)	1 (2)	7 (11)
Malignant lymphoma	4 (8)	4 (8)	9 (18)	7 (11)

1 Data taken from tables A1 and B1 of study report.
2 Numbers in parentheses indicate percent incidence
* $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$

No treatment-related increases in tumor incidence were observed in this study. Neoplastic lesions occurring frequently in a non-treatment-related incidence included alveolar carcinoma, hepatic adenoma and carcinoma and malignant lymphoma. The increased incidence of malignant lymphoma at 33 ppm was not statistically significant.

E. DISCUSSION:

Administration of methyl bromide vapor to $B6C3F_1$ mice by inhalation exposure for 20 weeks resulted in severe toxicity at 100 ppm (0.3876 mg/l), especially in males. Survival was sharply reduced in males, with a sudden decrease occurring between Weeks 12 and 20 at 100 ppm. Both males and females showed decreased body weight/body weight gain. Despite termination of exposure of high-dose mice to methyl bromide after week 20, clinical signs such as tremors, ataxia, abnormal posture and limb paralysis often persisted until termination. The persistence of these signs was probably due to degenerative microscopic lesions in the cerebrum and cerebellum induced by prolonged exposure to methyl bromide at 100 ppm.

The major target organs in mice following exposure to methyl bromide at 100 ppm in this study were the brain, heart, sternum and olfactory epithelium. Degenerative or dysplastic lesions were observed in all of these affected organs. The study author noted that the types

of microscopic lesions were generally different in animals that died early in the study from those that survived at least 1½ years. For example, early death animals tended to show cardiac degeneration, whereas those surviving or dying later in the study developed chronic cardiomyopathy. Dysplasia of the olfactory epithelium developed with time, whereas necrosis was observed primarily in early death animals, suggesting that cytotoxicity together with local irritation caused dysplasia with prolonged exposure. Lesions were qualitatively similar among males and females although males generally appeared to be more sensitive to development of lesions associated with shorter-term exposure.

No significant toxicity was observed at 10 or 33 ppm in either males or females, although a marginal reduction in body weight gain (-12%) was observed in females at Week 100 and abnormal posture was observed late in the study in 1 male and 3 females. Due to the low incidence of the clinical effects and the small decrease in gain, these effects were not considered to be significant treatment-related effects by TB-I or the study author. An intermediate dose group was not introduced following discontinuation of the high dose exposure. The results of this study indicate that methyl bromide has a steep time-dose response curve, especially in male mice, with no significant toxicity at 33 ppm but high mortality in males, body weight decreases and pronounced neurologic effects at 100 ppm.

There were no significant treatment-related increases in neoplastic lesions in males or females at any dose tested. Despite problems with the dose selection and non-conformity of the study with some of the guideline requirements (see study deficiencies, below), this study is considered adequate for regulatory purposes based on the steep dose- and time-dependent mortality curve. The data suggest that the mid-dose and NOEL of 33 ppm may approach a chronically toxic exposure.

This study was peer-reviewed by outside experts prior to publication according to the procedures of the National Toxicology Program and was determined to be adequate for assessment of carcinogenicity of methyl bromide in mice from long-term inhalation exposure. Methyl bromide was classified as a Group D carcinogen (no evidence for carcinogenicity in humans) by the NTP Peer Review Committee.

F. <u>STUDY DEFICIENCIES</u> are as follows:

For Carcinogenicity:

- results of gross examination not reported;
- MTD exceeded in high dose; no overt toxicity in mid-dose.

For Chronic Toxicity:

- clinical chemistry evaluation not performed;
- urinalysis not performed;
- food consumption not recorded;
- gross examination results not reported.

011243

Guideline Series 84: MUTAGENICITY

EPA Reviewer: Linnea J. Hansen, Ph.D
Review Section IV, Toxicology Branch I (7509C)
EPA Secondary Reviewer: Irving Mauer, Ph.D.
Toxicology Branch I (7509C)

Linnea Hansen, Date 9/7/94
Irving Mauer, Date 09/07/94
Nc 9/21/94

DATA EVALUATION REPORT

STUDY TYPE: Mutagenicity: Salmonella/Mammalian Activation Gene Mutation Assay, Mouse Bone Marrow Sister Chromatid Exchange Assay and Mouse Peripheral Blood Cell Micronucleus Assay; studies conducted as part of the Toxicity and Carcinogenicity Testing of Methyl Bromide in Mice, National Toxicology Program (not submitted to fulfill mutagenicity guideline requirements).

TOX. CHEM. NO: 555

P.C.CODE.: 053201

MRID NO.: 425041-01

TEST MATERIAL: Methyl bromide

SYNONYMS: Bromomethane

STUDY NUMBER: Laboratory Project Identification NTP TR 385

SPONSOR: Methyl Bromide Industry Panel

TESTING FACILITY: National Toxicology Program, Brookhaven National Laboratories, Integrated Laboratory Systems, Experimental Pathology Laboratories and Biotechnology Services, Inc.

TITLE OF REPORT: Methyl Bromide: Toxicology and Carcinogenesis Studies of Methyl Bromide in B6C3F, Mice (Inhalation Studies)

AUTHOR: S.L. Eustis, D.V.M.

REPORT ISSUED: March, 1992

EXECUTIVE SUMMARY: In a series of genotoxicity tests conducted as part of the

National Toxicology Program's toxicity and carcinogenicity testing of methyl bromide in mice (NTP Technical Report No. 385), a Salmonella reverse gene mutation assay, mouse bone marrow sister chromatid exchange assay and a mouse peripheral blood cell micronucleus assay were performed.

Salmonella Reverse Mutation Assay: Strains TA98 and TA100, with or without metabolic activation, were exposed using a desiccator protocol to methyl bromide vapor at 11 concentrations between 0.004 and 2.400 moles/L. Exposure was for 48 hrs at 37°C. Triplicate plates were run for each dose and a total of 3 trials were conducted with 2 - 5 doses of methyl bromide each. Negative controls (air only) and standard positive controls were included with each trial.

Methyl bromide caused an increase in revertants/plate (3.5 to 6-fold over negative controls) in strain TA100 with or without metabolic activation, but was negative in strain TA98. Cytotoxicity was observed at doses at or above 0.900 moles/L; slight cytotoxicity was sometimes observed between 0.120 - 0.600 moles/L. Positive controls caused increased revertants/plate in each strain, with and without metabolic activation.

Mouse Bone Marrow Sister Chromatid Exchange Assay: Male and female B6C3F_1 mice were exposed by inhalation to methyl bromide vapor 6 hrs/day for 5 days/week, for either 14 days at 0, 12, 25, 50, 100 or 200 ppm (0, 0.0463, 0.09257, 0.1938, 0.3876 or 0.7751 mg/l, respectively) or 12 weeks at 0, 10, 20, 40, 80 or 120 ppm (0, 0.03876, 0.1550, 0.3100 or 0.4651 mg/l, respectively). Bone marrow cells taken from the femurs were examined for the number of SCEs/cell.

Methyl bromide caused a slight increase in SCEs in male and female mice in the 14-day study at all exposure levels, especially at 200 ppm in females (increase of 2 SCE/cell). SCE were not induced in the 12-week study. Cell cycle kinetics and PCE:NCE ratio were not affected. Dosing was considered adequate based on signs of toxicity at or above 100 ppm.

Mouse Peripheral Blood Cell Micronucleus Assay: Four male and 4 female B6C3F_1 mice were exposed by inhalation to methyl bromide vapor for 6 hrs/day, 5 days/week over 14 days at 0, 12, 25, 50, 100 or 200 ppm (0, 0.0463, 0.09257, 0.1938, 0.3876 or 0.7751 mg/l, respectively), or over 12 weeks at 0, 10, 20, 40, 80 or 120 ppm (0, 0.03876, 0.1550, 0.3100 or 0.4651 mg/l, respectively). Stained and fixed slides of peripheral blood cells were prepared after termination of exposures and cells were examined for micronucleus formation.

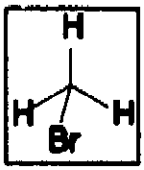
Methyl bromide caused increased formation of micronuclei in mice exposed in the 14-day study compared to negative controls, especially at 100 and 200 ppm (3 to 5.3-fold increase, respectively), but not in the 12-week study. Cell cycle kinetics and PCE:NCE ratio were not affected. Dosing was considered adequate based on signs of toxicity at or above 100 ppm.

These mutagenicity studies are core-supplementary but were not submitted to satisfy guideline requirements. The mutagenicity data requirements have been satisfied and these studies were reviewed by TB-I as additional information included in the NTP mouse cancer bioassay submitted for Guideline 83-2b (mouse carcinogenicity). The studies demonstrate that methyl bromide may have mutagenic potential for both gene mutation and chromosomal effects.

These studies were not submitted to fulfill guideline requirements for mutagenicity. The studies are considered Core-supplementary and were submitted as part of the NTP toxicity and carcinogenicity studies on methyl bromide and reviewed to provide additional data on the mutagenic potential of methyl bromide.

A. MATERIALS:

1. Test Material: Methyl Bromide, technical
   Description: colorless, odorless gas
   Lot/Batch #: E21-1012-00 (Matheson Gas Products, Joliet, IL)
   Purity: 99.8% a.i.
   Stability of compound: stable stored under pressure at room temperature
   CAS #: 74-83-9
   Structure



2. Test animals: Species: Mouse
   Strain: $B6C3F_1$
   Age and weight at study initiation: 6 weeks/
   Source: Frederick Cancer Research Facility, Frederick, MD
   Housing: Individually, stainless steel wire cages
   Environmental conditions: Temperature: 65 - 82°F
   Humidity: 11 - 85%
   Air changes: 15/hr
   Photoperiod: 12 hr light/12 hr dark
   Acclimation period: 2 weeks including 8 day quarantine

3. Test Atmosphere Generation and Analysis: Mouse Sister Chromatid Exchange and Micronucleus Assays

   Exposure Chambers and Generation of Test Atmosphere: Mice were exposed to methyl bromide vapor or chamber air into 1.4 m^3 stainless steel and glass or lucite exposure chambers. Vapor was delivered from compressed gas cylinders via a shrouded delivery tube to a distribution plenum. Rotameters were used to control gas flow to each chamber. Chambers reached 90% of target concentration within 20

minutes of initiation of delivery. Chamber atmospheres were exhausted from the bottoms of the chambers through charcoal filters.

Chamber concentrations of methyl bromide were measured using a MIRAN 80 infrared spectrophotometer. Air samples from each chamber were removed for analysis hourly (for 10 minutes) during exposures. Uniformity of vapor distribution in each chamber was measured from 8 ports. The study report did not indicate whether oxygen concentration was determined during the study.

Results - The 10 ppm chamber concentration measurements varied from 90 - 120% of target concentration during the first week of exposure; thereafter all but a few measurements were within 110% of target. At 33 and 100 ppm, all measurements were within 10% of target. Spatial variation of the distribution of methyl bromide vapor in each chamber did not exceed 4.2%. The occasional variations observed in this study at 10 ppm do not affect the results of this study.

3. Animals received food (NIH-07 Mouse Ration, Zeigler Bros., Inc.) and tap water ad libitum, except during inhalation exposures.

B. MUTAGENICITY TEST PERFORMANCE AND RESULTS

Salmonella Reverse Gene Mutation Assay

Method: A desiccator protocol (technique of Zeiger, 1990) was used to expose bacterial cultures to methyl bromide vapor. Salmonella strain TA98 or TA100, with or without S9 mix (30%; prepared from livers of Sprague-Dawley rats or Syrian golden hamsters treated with Arochlor 1254), were prepared on minimal glucose agar plates. Plates were exposed to methyl bromide vapor in glass desiccator jars which were sealed and partially evacuated, then injected with air and methyl bromide vapor to give the appropriate concentrations. Cultures were exposed at 37°C for 48 hrs to concentrations of 0.000, 0.004, 0.012, 0.040, 0.120, 0.300, 0.400, 0.600, 0.900, 0.980, 1.200 or 2.400 moles methyl bromide/L. Triplicate plates were exposed at each concentration, along with triplicate plates of concurrent positive control (2-aminoanthracine, with activation; 4-nitro-o-phenylenediamine, TA98 without activation; sodium azide, TA100 without activation) and negative control (air only). The concentrations of positive control materials tested was not indicated in the study report. No further details on materials or experimental protocol were provided.

Results: Methyl bromide was mutagenic in strain TA100 with or without metabolic activation. A 3.5 to 6-fold increase in the number of revertants compared to controls was observed at all doses tested. A dose-response was not observed. Methyl bromide was not mutagenic in strain TA98. Cytotoxicity was observed at 0.900 moles/L and slight toxicity was occasionally observed at 0.120 - 0.600 moles/L. Positive controls showed significant increases in the number of revertants/plate in all trial groups, with and without S9

activation.

Mouse Bone Marrow Sister Chromatid Exchange Assay

Methods: Five B6C3F$_1$ mice/dose/sex were exposed to methyl bromide for 14-days at 0, 12, 25, 50, 100 or 200 ppm (0, 0.0463, 0.09257, 0.1938, 0.3876 or 0.7751 mg/l, respectively) or 12 weeks at 0, 10, 20, 40, 80 or 120 ppm (0, 0.03876, 0.1550, 0.3100 or 0.4651 mg/l, respectively); (6 hr exposure/day, 5 exposure days/week; see mouse carcinogenicity study DER for additional information on short-term inhalation studies in mice). Subcutaneous implantations of 50 mg bromodeoxyuridine (BrdU) were administered at 24 hrs pre-sacrifice, followed by an intraperitoneal injection of 2 mg/kg colchicine at 2 hrs pre-sacrifice. Marrow from one or both femurs was collected in PBS· pH 7.0, lysed with hypotonic salt solution, fixed and stained using the fluorescence-plus-Giemsa method. For each animal, 25 second-division metaphase cells were scored, with the number of SCEs per cell noted. One hundred additional cells/animal were scored to measure cell cycle kinetics. Data were transformed (square-root) and analyzed by one-way ANOVA with regression tests and student's t-test used for pairwise comparison to controls. No further details were provided.

Results: Results of the SCE assay in mouse bone marrow are shown in Tables F2 and F3 of the study report, appended to this DER. SCE were induced in bone marrow cells of female mice following the 14-day period at the highest dose tested (increase of 2 SCE/cell at 200 ppm compared to controls) but not after 12-weeks' exposure. In males, only a slight increase (1 SCE/cell) was observed at 200 ppm.

Dosing was adequate for both the 14-day and 12-week study, based on signs of toxicity, including mortality in males, observed at the highest doses in each study. Additional information on toxicity observed in these studies is contained in the appendix to the DER of the NTP mouse carcinogenicity study on methyl bromide.

Mouse Peripheral Blood Micronucleus Assay

Methods: Peripheral blood was collected from 4 B6C3F$_1$ mice/exposure group from the 14-day study on Day 14 and from the 13-week study on methyl bromide at 4, 8 and 12 weeks (see mouse carcinogenicity DER for details on exposures). Mice were exposed to 0, 12, 25, 50, 100 or 200 ppm (0, 0.0463, 0.09257, 0.1938, 0.3876 or 0.7751 mg/l, respectively) for 14 days and to 0, 10, 20, 40, 80 or 120 ppm (0, 0.03876, 0.1550, 0.3100 or 0.4651 mg/l, respectively) for up to 12 weeks; 6 hrs/day and 5 days/week. Smears were prepared on glass slides, fixed in 100% methanol and stained with acridine orange. Stained and coded slides were examined for polychromatic and normochromatic erythrocytes (PCEs and NCEs). A total of 1000 RBCs per animal were scored for micronuclei. Micronuclei were defined by the criteria of Schmid (1976) and data was analyzed using Kruskal-Wallis one-way ANOVA and the Mann-Whitney U test. No further details were provided.

Results: Results of the micronucleus test are shown in Tables F2 and F3 of the study report, appended to this DER.

14-day exposure - Methyl bromide induced micronuclei at all doses tested in female mice, but particularly at 100 and 200 ppm (3 and 5.3-fold increase compared to controls, respectively). Increases at lower doses were less pronounced (1.5 to 2.3-fold). Results in males were considered equivocal since the increase was slight and not dose-related. Average generation time (AGT) and PCE:NCE ratio were not affected in either males or females.

4 - 12 week exposures - Methyl bromide did not induce micronuclei in peripheral blood cells of $B6C3F_1$ mice and did not affect cell cycle kinetics (no change in AGT). Methyl bromide did not affect the PCE:NCE ratio.

Dosing was adequate for both the 14-day and 12-week study, based on signs of toxicity, including mortality in males, observed at the highest doses in each study. Additional information on toxicity observed in these studies is contained in the appendix to the DER of the NTP mouse carcinogenicity study on methyl bromide.

DISCUSSION/CONCLUSIONS

Methyl bromide was positive in three types of mutagenicity assays under the conditions of testing. The results of this study are consistent with previously published studies which demonstrated genotoxicity of methyl bromide in numerous mutagenicity assays, including Salmonella reverse gene mutation assays and sister chromatid exchange in human peripheral lymphocytes.

The positive effects were observed only in mice exposed to methyl bromide vapor over 14 days; mice exposed for 4 - 12 weeks showed no effects. The reason for the inconsistent results obtained from different exposure times is unclear. The effects seen in the 14-day study were not due to testing at higher dose levels than the 12-week study. One possibility suggested by the study author is that metabolism may be altered with prolonged exposure, resulting in either less target cell exposure or reduced target cell sensitivity.

With the exception of a specific locus test in mouse which was recently requested by the Agency because of positive results in a rat testicular DNA alkaline elution assay, the mutagenicity data requirements have been satisfied for methyl bromide. The studies reviewed in this DER are considered supplemental information and support the conclusion that methyl bromide may have genotoxic potential.

TABLE F1
Mutagenicity of Methyl Bromide in *Salmonella typhimurium*[a]

Strain	Dose (moles/L)	Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3
		-S9			+30% hamster S9		
TA100	0.000	166 ± 6.8	166 ± 6.9	128 ± 12.7	115 ± 9.0	162 ± 3.2	178 ± 9.8
	0.004			518 ± 24.4			796 ± 5.4
	0.012			510 ± 20.7			632 ± 3.1
	0.040			430 ± 5.2			577 ± 12.0
	0.120		602 ± 6.1	3 ± 0.9[b]		871 ± 8.7	14 ± 1.5[b]
	0.300	675 ± 19.2			600 ± 27.5		
	0.400		Toxic			0 ± 0.0[b]	
	0.600	2 ± 0.7[b]			4 ± 0.3[b]		
	0.800	Toxic			Toxic		
	0.920		Toxic			Toxic	
	1.200	Toxic	Toxic		Toxic	Toxic	
	2.400	Toxic			Toxic		
Trial summary		Positive	Positive	Positive	Positive	Positive	Positive
Positive control[c]		621 ± 10.2	363 ± 11.9	324 ± 3.0	539 ± 25.1	736 ± 22.2	516 ± 17.3

TA100 (continued)

Dose (moles/L)	Trial 1	Trial 2	Trial 3
	+30% rat S9		
0.000	140 ± 9.2	172 ± 11.9	190 ± 11.0
0.004			786 ± 34.4
0.012			670 ± 55.2
0.040			992 ± 24.5
0.120		811 ± 3.2	21 ± 2.7[b]
0.300	567 ± 32.1		
0.400		0 ± 0.0[b]	
0.600	13 ± 2.5[b]		
0.800	Toxic		
0.920		Toxic	
1.200	Toxic	Toxic	
2.400	Toxic		
Trial summary	Positive	Positive	Positive
Positive control	1,026 ± 85.1	701 ± 1.5	920 ± 45.0

TABLE F1
Mutagenicity of Methyl Bromide in *Salmonella typhimurium* (continued)

Strain	Dose (nmole/L)	Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3
		-S9			+30% hamster S9		
TA98	0.000	13 ± 1.2	26 ± 3.7	25 ± 2.9	26 ± 3.2	33 ± 3.2	29 ± 3.7
	0.004			14 ± 1.9			21 ± 3.0
	0.012			13 ± 1.2			16 ± 4.1
	0.040			3 ± 0.6			9 ± 1.7
	0.120		8 ± 1.8	0 ± 0.0[a]		10 ± 1.2	1 ± 0.6[a]
	0.360	8 ± 0.7			9 ± 2.5		
	0.600		0 ± 0.0[a]			0 ± 0.0[a]	
	0.800	Toxic			0 ± 0.3[a]		
	0.900	Toxic			Toxic		
	1.000		Toxic			Toxic	
	1.200	Toxic	Toxic		Toxic	Toxic	
	2.400	Toxic			Toxic		
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		265 ± 5.1	209 ± 2.7	310 ± 3.5	314 ± 21.4	409 ± 23.8	393 ± 14.5

TA98 (continued)

Dose (nmole/L)	Trial 1	Trial 2	Trial 3
	+30% rat S9		
0.000	25 ± 3.9	28 ± 4.7	32 ± 1.2
0.004			17 ± 1.7
0.012			17 ± 2.0
0.040			10 ± 3.0
0.120		11 ± 0.7	2 ± 0.3[a]
0.360	11 ± 1.5		
0.600		0 ± 0.0[a]	
0.800	1 ± 0.7		
0.900	Toxic		
1.000		Toxic	
1.200	Toxic	Toxic	
2.400	Toxic		
Trial summary		Negative	Negative
Positive control	372 ± 15.5	339 ± 16.0	284 ± 19.7

Negative

[a] Study performed by Microbiological Associates. A description of the desiccator protocol is presented in Zeiger (1990); modifications are described in the Protocol section of this appendix. Cells and methyl bromide or carrier (air) were incubated in the absence of exogenous metabolic activation (-S9) or with Aroclor 1254-induced S9 from male Syrian hamster liver or male Sprague-Dawley rat liver. High dose was limited by toxicity. 0 nmole/L dose is the control.
[b] Revertants are presented as means ± standard error from three plates.
[c] Slight toxicity
Positive control: 2-aminoanthracene was used on all strains in the presence of S9. In the absence of metabolic activation, 4-nitro-o-phenylenediamine was tested on TA98 and sodium azide was tested on TA100.

TABLE F3
Cytogenetic and Micronuclei Data for Mice in the 14-Day Inhalation Studies of Methyl Bromide[a]

Dose	No. of SCEs[b]	Average Generation Time[c]	Micronuclei/ 1,000 Erythrocytes[c]
Male			
0 ppm	3.7 ± 0.3	13.4 ± 0.1	5.6 ± 0.5
12 ppm	3.4 ± 0.1	12.6 ± 0.2	5.4 ± 0.9
25 ppm	4.3 ± 0.3	12.7 ± 0.4	7.7 ± 1.9
50 ppm	4.4 ± 0.1	12.4 ± 0.2	6.4 ± 1.2
100 ppm	4.3 ± 0.2	12.6 ± 0.2	7.2 ± 1.0
200 ppm[d]	4.8	12.2	4.0
	P=0.021	P=0.224	P=0.622
Female			
0 ppm	3.2 ± 0.2	13.1 ± 0.4	3.0 ± 0.4
12 ppm	3.8 ± 0.5	12.8 ± 0.5	7.0 ± 1.2
25 ppm	3.6 ± 0.3	11.9 ± 0.1	5.0 ± 0.8
50 ppm	3.3 ± 1.1	12.2 ± 0.1	4.5 ± 0.5
100 ppm	4.8 ± 0.1	12.0 ± 0.2	9.0 ± 0.8
200 ppm[e]	5.3 ± 0.1	12.3 ± 0.1	16.0 ± 1.2
	P=0.003	P=0.178	P=0.001

[a] Data presented as mean ± standard error. P values are for trend tests as indicated in the Pretest section of this appendix. Significance occurs at P≤ 0.05.
[b] Four animals per dose group; 25 cells scored per animal.
[c] Five animals per dose group; 100 cells scored per animal for average generation time determination, and 1,000 erythrocytes per animal scored for micronuclei.
[d] Only one animal survived at this dose level. Data are presented but were not used for statistical purposes.
[e] One animal died at this dose level.

TABLE F3
Cytogenetic and Micronuclei Data for Mice in the 12-Week Inhalation Studies of Methyl Bromide[a]

Analysis	0 ppm	10 ppm	30 ppm	40 ppm
Male				
Sister chromatid exchange	4.2 ± 0.13[b]	3.6 ± 0.30[b]	4.1 ± 0.26[b]	4.0 ± 0.39[b]
Average generation time	12.1 ± 0.15[b]	12.3 ± 0.08[b]	12.0 ± 0.14	12.5 ± 0.14
Micronuclei/normochromatic erythrocyte				
4 weeks	3.7 ± 0.71[c]	3.0 ± 0.68[d]	3.5 ± 0.42	2.8 ± 0.49
8 weeks	1.8 ± 0.48[d]	2.7 ± 0.30[c]	2.5 ± 0.54	1.3 ± 0.70
12 weeks	3.2 ± 0.48[b]	2.9 ± 0.69[d]	2.0 ± 0.33	3.3 ± 0.48
Polychromatic erythrocytes (%)				
4 weeks	4.69 ± 1.196[c]	2.64 ± 0.377[d]	2.75 ± 0.256	2.74 ± 0.533
8 weeks	3.85 ± 0.649[d]	2.57 ± 0.475[c]	2.63 ± 0.414	2.83 ± 0.673
12 weeks	2.92 ± 0.497[b]	3.21 ± 0.383[d]	2.86 ± 0.338	4.36 ± 0.774
Micronuclei/polychromatic erythrocyte				
4 weeks	2.9 ± 0.46[c]	1.9 ± 0.66[d]	1.6 ± 0.32	1.5 ± 0.50
8 weeks	2.5 ± 0.76[d]	1.4 ± 0.37[c]	2.8 ± 0.70	1.5 ± 0.60
12 weeks	1.8 ± 0.46[b]	2.1 ± 0.46[d]	1.1 ± 0.40	2.6 ± 0.45
Female				
Sister chromatid exchange	4.4 ± 0.65[b]	5.6 ± 0.19[b]	4.9 ± 0.33[b]	4.2 ± 0.28[b]
Average generation time	12.4 ± 0.16	12.3 ± 0.19	12.3 ± 0.09	12.3 ± 0.16
Micronuclei/normochromatic erythrocyte				
4 weeks	1.5 ± 0.19	2.5 ± 0.42	1.8 ± 0.31	1.8 ± 0.25
8 weeks	1.6 ± 0.38	1.8 ± 0.37	1.6 ± 0.46	1.9 ± 0.30
12 weeks	2.3 ± 0.41	3.4 ± 0.89	3.4 ± 0.42	1.8 ± 0.25
Polychromatic erythrocytes (%)				
4 weeks	2.26 ± 0.345	2.71 ± 0.387	2.46 ± 0.215	2.40 ± 0.445
8 weeks	3.21 ± 0.312	4.38 ± 0.659	3.58 ± 0.471	3.31 ± 0.524
12 weeks	2.04 ± 0.283	2.36 ± 0.286	2.70 ± 0.246	1.99 ± 0.277
Micronuclei/polychromatic erythrocyte				
4 weeks	1.8 ± 0.31	2.0 ± 0.63	1.8 ± 0.27	0.6 ± 0.18
8 weeks	1.3 ± 0.16	1.3 ± 0.31	1.4 ± 0.50	1.6 ± 0.32
12 weeks	0.9 ± 0.30	1.6 ± 0.58	1.4 ± 0.58	0.8 ± 0.16

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TABLE F3
Cytogenetic and Micronuclei Data for Mice in the 13-Week Inhalation Studies of Methyl Bromide[a] (continued)

Analysis	0 ppm	80 ppm	120 ppm
Male (continued)			
Sister chromatid exchange	4.2 ± 0.15[b]	3.9 ± 0.28[b]	4.1 ± 0.33[b]
Average generation time	12.1 ± 0.15[b]	12.2 ± 0.07	12.4 ± 0.16
Micronuclei/normochromatic erythrocyte			
4 weeks	3.7 ± 0.71[c]	1.4 ± 0.30[c,**]	2.6 ± 0.42[c]
8 weeks	1.8 ± 0.48[d]	2.1 ± 0.61	1.9 ± 0.39
12 weeks	3.2 ± 0.48[c]	2.3 ± 0.37	3.6 ± 0.32
Polychromatic erythrocytes (%)			
4 weeks	4.89 ± 1.196[c]	4.65 ± 0.874	3.44 ± 0.468
8 weeks	3.85 ± 0.469[d]	3.63 ± 0.953	2.94 ± 0.551
12 weeks	2.92 ± 0.497[c]	3.24 ± 0.374	3.39 ± 0.511
Micronuclei/polychromatic erythrocyte			
4 weeks	2.9 ± 0.49[c]	1.9 ± 0.55	2.9 ± 0.55
8 weeks	2.5 ± 0.78[d]	1.1 ± 0.35	2.1 ± 0.35
12 weeks	1.8 ± 0.49[c]	1.5 ± 0.19	2.3 ± 0.75
Female (continued)			
Sister chromatid exchange	4.4 ± 0.65[b]	4.8 ± 0.31[b]	5.1 ± 0.21[b]
Average generation time	12.4 ± 0.16	12.2 ± 0.18	12.2 ± 0.12
Micronuclei/normochromatic erythrocyte			
4 weeks	1.5 ± 0.19	0.8 ± 0.25	2.5 ± 0.46
8 weeks	1.6 ± 0.38	1.8 ± 0.41	1.5 ± 0.42
12 weeks	2.3 ± 0.41	1.9 ± 0.30	1.8 ± 0.37
Polychromatic erythrocytes (%)			
4 weeks	2.26 ± 0.345	2.16 ± 0.283	2.33 ± 0.158
8 weeks	3.21 ± 0.312	2.85 ± 0.482	3.90 ± 0.425
12 weeks	2.04 ± 0.283	3.39 ± 0.339*	2.69 ± 0.316
Micronuclei/polychromatic erythrocyte			
4 weeks	1.8 ± 0.31	0.9 ± 0.23	1.6 ± 0.42
8 weeks	1.3 ± 0.16	1.0 ± 0.27	1.0 ± 0.33
12 weeks	0.9 ± 0.30	1.4 ± 0.32	1.6 ± 0.32

* Significantly different ($P \le 0.05$) from the control group
** $P \le 0.01$
a Mean ± standard error; four animals per dose group analyzed for SCE and AGT; eight animals per dose group analyzed for MN
b Except where noted
c n=6
d n=8
e n=4
f n=7

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END